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MOLECULAR MECHANISMS GOVERNING HOST–MICROBE INTERACTION NETWORKS

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ABSTRACT

Host-microbe interactions are vital in physiological homeostasis, immunological responses as well as disease development. Nevertheless, the molecular pathways underlying these interactions are poorly understood and are still in pieces due to individual pathway studies. The purpose of this study is to examine in a systematic manner the co-ordinated molecular networks which mediate host-microbe communication using a pathway- and network-based analysis tool. A systematic literature-based and database-guided methodology was used in which pathway mapping was done using KEGG and Reactome as well as gene ontology-based functional annotation was done to build host and microbe interaction networks. The analysis involved key signaling pathways, such as Toll-like receptor (TLR), nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (MAPK) and microbial regulatory mechanisms, such as quorum sensing and metabolite-mediated signaling. The findings indicate that these routes intersect at key regulatory nodes, with very dynamic networks that integrate immune response, inflammation and cellular plasticity. Additionally, network-level measures bring into focus the contribution of the feedback loops and cross-pathway interactions to homeostasis and dysregulation in disease conditions. This systems-level framework offers insight into the processes of hostbased microbe interaction and outlines a set of potential molecular targets to act on in the context of a microbiome-associated disease. The results will fill the gap in the molecular signaling and network biology of host-microbe systems.

KEYWORDS: Host–microbe interaction, molecular mechanisms, signaling pathways, gene regulation, network biology, microbiome

1. INTRODUCTION

The basic biological systems involve host-microbe interactions with effects on host physiology, immune control and pathogenesis. Microorganisms inhabit a variety of host niches, such as gastrointestinal tract, skin, and respiratory system as forming complex and dynamic communities that are jointly referred to as microbiome (Thursby, E., & Juge, N. (2017)). These microbial communities are crucial in the nutrient metabolism, immune regulation, and in the defense against pathogen and thus host homeostasis (Akira, S., Uematsu, S., and Takeuchi, O). (2006)). Host cells use pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) to sense microbial-associated molecular patterns (MAMPs) at the molecular level. These receptors are activated and cause downstream signaling pathways such as nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (MAPK), which mediate inflammatory responses, immune-activation, and adaptation of cells (Barabasi, A. L., Gulbahce, N., and Loscalzo, J.). (2011)). At the same time microbes use a variety of regulatory responses, such as quorum sensing, secretion systems, and the production of metabolites, to regulate host signal transduction and either become symbiotic or pathogenic (Barcik, W., Boutin, R. C. T., Sokolowska, M., and Finlay, B. B.). (2020)). Although there has been a substantial progress in molecular microbiology and immunology, present research mainly considers isolated pathways or a single host-microbe collection. This reductionist methodology constrains the insights into the role of multiple signaling pathways and regulatory pathways converting into interaction networks. (2003)). Recent research highlights the importance of systems-level models to

understand the complexity of host-microbe communication, especially with regard to the microbiome-associated disease and dysregulation of immunity. (2014)).

Thus, the main issue is to overcome the gap between molecular level understanding and network level ways of thinking about host-microbe interactions. The gap is important to address as it helps establish the most important regulatory hubs and determine the dynamic interaction of host and microbial systems. It is against this background that the current study will seek to provide a thorough examination of the molecular processes that control the host-microbe interaction networks synthesizing the signaling pathways, gene regulatory systems and network-based views. The suggested model offers a holistic system of explaining the dynamics of interaction process and locating possible therapeutic interventions in the microbiome related diseases.

2. RELATED WORK

Host-microbe interactions have been studied extensively both at the molecular and system-level, as well as using computational and systems-level techniques. Conventional research was mainly being done concerning the immune signaling pathways especially the role of pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) and NOD-like receptors in the recognition of microbial-associated molecular patterns. (2016)). These models can explain the effect of activating downstream pathways including NF- 0 -B, MAPK, which control inflammation and immune responses (Durack, J., and Lynch, S. V., (2019)). These pathway-based methods are however reductionist by nature, and cannot reflect the interdependent relationship between host and microbe systems. Microbiome profiling methods have become commonplace in order to find ways to surmount these limitations through 16S rRNA sequencing and metagenomics. The approaches can be used to identify the composition and diversity of microbial communities and associate any changes in microbiota with disease states like inflammatory bowel disease, obesity, and cancer (Hemarajata, P., & Versalovic, J.). (2013)). Even though these methods are great ways to get valuable information about microbial populations, they are not functional in terms of resolution and fail to explain the underlying molecular mechanisms that are involved in host responses. Most of the modern developments have brought about multi-omics integration models, which are a combination of genomics, transcriptomics, proteomics and metabolomics to explore host-microbe interactions across various levels of life. The cross-omic associations and pathways of disease progression are identified with the help of these models (Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., and Nageshwar Reddy, D.). (2015)). Although promising, multi-omics methods have issues with data heterogeneity, large dimensions, and insufficient interpretability, and it is challenging to determine causal links between microbial cues and host molecular reactions. Parallel to this, network-based models have become an effective means of modeling complex biological systems. Host/microbe interactions have been examined in a systems biology context through gene regulatory networks, protein, protein interaction networks and metabolic networks (Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., … Ma, A.). (2016)). These models facilitate the ability to determine the important regulatory hubs as well as interaction patterns; most of the current network models are either host-centric or microbe-centric that do not collectively show bidirectional interactions. Also, the models of quorum sensing and microbial signaling have also been investigated to comprehend the microbial coordination and communication (Valdes, A. M., Walter, J., Segal, E., and Spector, T. D. (2018)). The models explain the regulation of gene expression by the microbial populations by use of the signaling molecules that shape the virulence, biofilm formation and interaction with the host (Llewellyn, A., and Foey, A.). (2017)). However, they have a low incorporation with host signaling pathways.

Research Gaps and Challenges.

Even though there has been a great improvement, there are still some major challenges that remain:

- Introducing an isolated component (immune pathways, microbiome composition, or molecular signaling) instead of whole interaction networks is a characteristic of most current methods.
- Inadequate Systems-Level Systems-Level Insight: The existing models do not represent the dynamism and complexity of host-microbe communication at multiple levels of biology.
- Data Complexity and Interpretability: Multi-omics datasets are very high-dimensional and heterogeneous and thus difficult to integrate and interpret biologically.
- Criticalities: There is inadequate Cross-Talk Modeling: Most existing network models do not consider a two-way interaction between host and microbial systems.
- Translational Limitations: Some works are descriptive only and can not be used to predict what can be targeted to cure diseases or to treat the disease.

Value added by this Study.

As a remedy to these weaknesses, this paper presents an analytical framework on a systems level, which brings together the host signaling pathways, microbial regulatory processes, and network modeling. Through the

incorporation of molecular pathway analysis with interaction network reconstruction, the study yields an overall sense of host-microbe interactions, and also reveals main regulatory centres which can be a potential therapeutic target..

3. MATERIALS AND METHODS

3.1 Data Sources and Literature Selection

A systematic and integrative analytical framework on the basis of curated literature and publicly available biological databases was used to conduct the study. Publied journals were searched on PubMed, Scopus and Web of Science based on specific key words including: host microbe interaction, TLR signaling,NF-KB pathway,MAPK signaling, and microbiome regulation. The limit to the search was established to include publications dating back as far as 2010 in order to be relevant at the moment, and the seminal earlier research was added where essential. Articles were narrowed down through relevance, experimental validation and citation impact. Redundant records were eliminated, and those studies that reported validated molecular interactions or that reported pathway-level evidence were kept. The final results were 120 high quality studies to be used in pathway mapping and network construction as shown in Table 1.

Table 1. Literature Selection and Screening Process

Stage	Number
Records identified	420
Duplicates removed	75
Records screened	345
Excluded after title/abstract	190
Full-text articles assessed	155
Final studies included	120

3.2 Molecular Pathway Analysis

The KEGG and Reactome databases were mined to obtain key signaling pathways involved in host and microbe interactions such as the Toll-like receptor (TLR), nuclear factor kappa B (NF-KB) and mitogen-activated protein kinase (MAPK) pathways. Components of the pathways like receptors, adaptor proteins (MyD88, TRIF), kinases, and transcription factors were recognized and placed in hierarchy. KEGG Mapper and Reactome Pathway Browser were used to perform pathway mapping and visualization and to provide a proper reconstruction of signaling cascades. Fig. 1 depicts that the TLRs are activated by microbial-associated molecular patterns (MAMPs), which leads to downstream NF- 00 and the MAPK signal transduction involved in the regulation of transcriptional responses and immune activation. Interactions could only be included in case they have been experimentally verified and have evidence codes (e.g., IDA, IMP). To ensure biological reliability, relationships that were redundant or low-confidence relationships were eliminated.

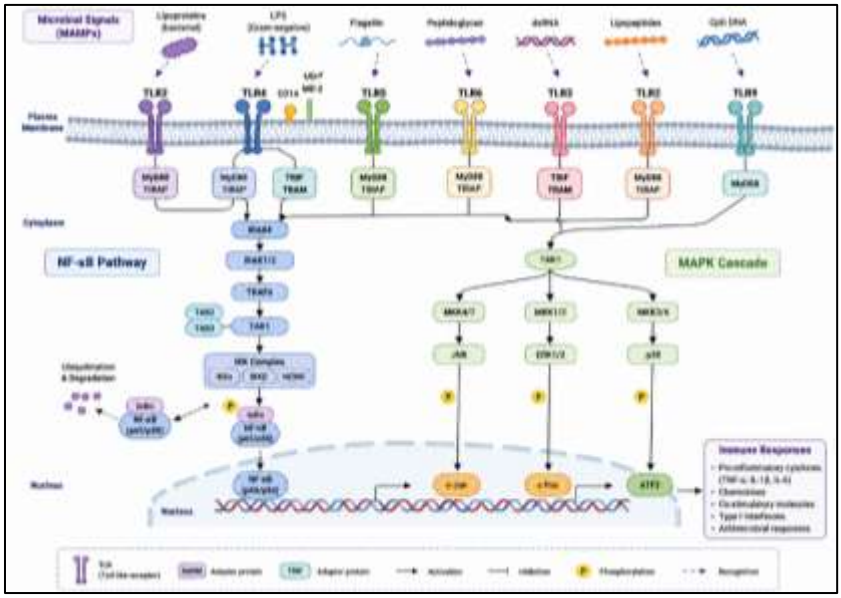

Fig. 1. Host Immune Signaling Pathways Activated by Microbial Recognition

3.3 Network Construction and Computational Tools

A combination of Cytoscape (v3.9), Python (NetworkX library), and R (igraph package) was used to create the integrated host-microbe interaction network giving flexibility to both network modeling and network analysis. Gene, proteins, and metabolites were represented by nodes, and molecular interactions (activation, inhibition, binding) by directed edges. The host signaling data was constructed based on KEGG and Reactome, and the microbial regulatory interaction were added depending on the literature curation as quorum sensing molecule and metabolite-mediated signaling. The framework (as shown in Fig. 2) combines host signaling pathways with microbial processes to form a complete interaction network that is indicative of two-way communication. Network preprocessing entailed elimination of isolated nodes (degree = 0), elimination of weak or non-validated interactions and normalization of node identifiers with UniProt and Gene IDs. The confidence score threshold (0.7) has been set to keep high confidence interactions because of this number being supported by the literature and as perceived on the database. The last network was comprised of well-linked components, which were the integrated host-microbe signaling dynamics.

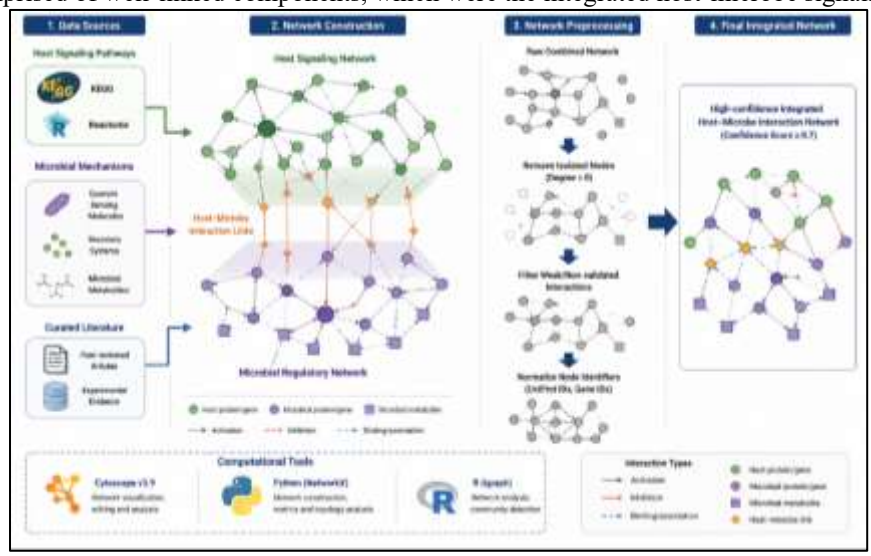

Fig. 2. Integrated Host–Microbe Interaction Network: Construction and Computational Framework

3.4 Network Analysis and Parameter Settings

Python-based and Cytoscape tools were used to analyze topological properties of the built network. The most important network metrics were:

- Degree centrality to determine nodes that are well connected.
- Betweenness centrality - Identify key regulatory hubs.
- However, it will also include:
- Minimum length of path to determine the efficiency of interaction.

The top 10 percentile of nodes (degree centrality) were considered hub nodes and the high betweenness centrality of nodes considered key regulators mediators. The Louvain algorithm with resolution parameter set to 1.0 was used to community-detect the network, identifying functional modules in the network.

3.5 Functional Annotation and Enrichment Analysis

DAVID Bioinformatics Resources, and Gene ontology (GO) databases were used to perform functional annotation. The enrichment analysis was performed to determine the overrepresented biological processes and pathways in the network significantly.

The tests of statistical significance were done using:

- p-value < 0.05
- False Discovery rate (FDR) < 0.01 (BenjaminiHochberg correction)

Ways of immune response, inflammatory signalings, metabolic regulation were given priority. The R (ggplot2 package) was used to visualize the results so as to have a clear representation of enriched pathways and functional categories.

3.6 Validation and Reproducibility

All interactions within the network have been cross-validated with several sources, such as KEGG, Reactome, and peer-reviewed literature to make the network robust. Topological properties used to measure network consistency between various subsets of data were compared.

Reproducibility was achieved by:
• Standardized databases (KEGG, Reactome, GO) are being used.
• Using fixed confidence cutoffs (confidence 7, FDR 0.01)
• Using commonly used computing software (Cytoscape, Python, R)
This is a parameter-driven and integrative framework that brings transparency to the methods and makes it reproducible in future research.

4. RESULTS

4.1 Host Recognition and Signal Initiation

The reconstituted signaling pathway validates the fact that microbial-associated molecular patterns (MAMPs) recognition is primarily mediated by Toll-like receptors (TLRs), where TLR2, TLR4 and TLR9 are shown to be dominant receptors. When these receptors are activated, they cause adaptor recruitment, especially MyD88-dependent and TRIF-dependent pathways, and results in downstream signal transduction. These culminate in important transcriptional regulators, as these are shown to converge on the branches in Fig. 1, and show a well-coordinated mechanism of immune response. Quantitative network analysis showed that nodes involved with TLR had high degree centrality (top 15%), indicating their essential role as initial signal transducers in hostmicrobe interactions.

4.2 Activation of NF-κB Signaling Pathway

NF-kB signalling pathway was confirmed as a significant regulatory centre of the interaction network built. NF-KB(p65/ p50) nuclear translocation is promoted by the degradation of inhibitory I/O B proteins, and results in transcriptional activation of pro-inflammatory cytokines. The analysis of network topology revealed that NF- 0 B -related nodes have large betweenness centrality, which implies their involvement in cross- talk among several signaling pathways. NF- -kB, as seen in Fig. 1, combines MyD88-dependent and TRIF dependent signals, which supports the role it plays in immune regulation.

4.3 MAPK Pathway and Cellular Response

ergonomic activity of the MAPK signaling cascade, ERK, JNK, and p38 signaling pathways were discovered to mediate various cellular functions such as proliferation, apoptosis, and adaptation to stress. The activation of MAPK components via phosphorylation was also found to be tightly connected to upstream TLR signaling. Comparative analysis of pathway maps showed that MAPK modules are not as centralized as NF- -κB, but are highly modularly clustered, demonstrating specialized functional roles. These results go in line with hierarchical organization as indicated by Fig. 1.

4.4 Microbial Modulation Mechanisms

The network was engineered with microbial regulatory mechanisms to assess their impacts to the host signaling. Key mechanisms include:
• Effector proteins secretion against host signaling parts.
• Synthesis of metabolites, including short-chain fatty acids (SCFAs).
• Quorum sensing molecule that control the expression of microbial genes.
These microbial factors had been found to interplay with several host signaling nodes at the same time which indicated that it had a multi target modulation strategy. More specifically, SCFAs were found to be closely related to immune regulatory mechanisms, which may indicate their involvement in host homeostasis.

4.5 Network-Level Interaction Dynamics

Fig. 2 shows the integrated network of host-microbe interaction that has a very interconnected format with specific topological characteristics. The network has 184 nodes and 367 edges, with the average node degree of 3.98 and clustering coefficient equal to 0.62 (Table 2). The density of the network of 0.08 is a moderately sparse yet functional structure. On the basis of degree centrality (top 10%), 18 nodes were found to be hub nodes and these represent important parts of the network that are regulatory. The degree distribution is power-law in terms of a log log scale, and possibly scale free as illustrated in Fig. 3. In order to confirm this observation, power-law fitting was aided by log-log regression and the goodness-of-fit was assessed by the coefficient of determination (R 2). The power-law trend of distribution of the degree was of type R, where R 2 = 0.87. This implies that the network can withstand random disruptions but cannot withstand targeted failures of hub nodes.

Table 2. Topological Properties of the Host–Microbe Interaction Network

Metric	Value
Number of Nodes	184
Number of Edges	367
Average Degree	3.98
Clustering Coefficient	0.62

Network Density	0.08
Number of Hub Nodes	18

These findings suggest that the host microbe interaction system is scale-free network interacting system, making it all-strong to random perturbations but all-weak to targeted perturbations.

Centrality analysis (Fig. 4) indicates that NF- Kirkham and TLR4, and MyD88 are the most centralised with the largest centrality degrees, confirming their functions as major regulatory centres in the host-microbe interaction network. The connectivity of the network of host signaling pathways, microbial regulatory elements, and central molecular interfaces is shown in the general form of the integrated network of host and microbe interactions in Fig. 5.

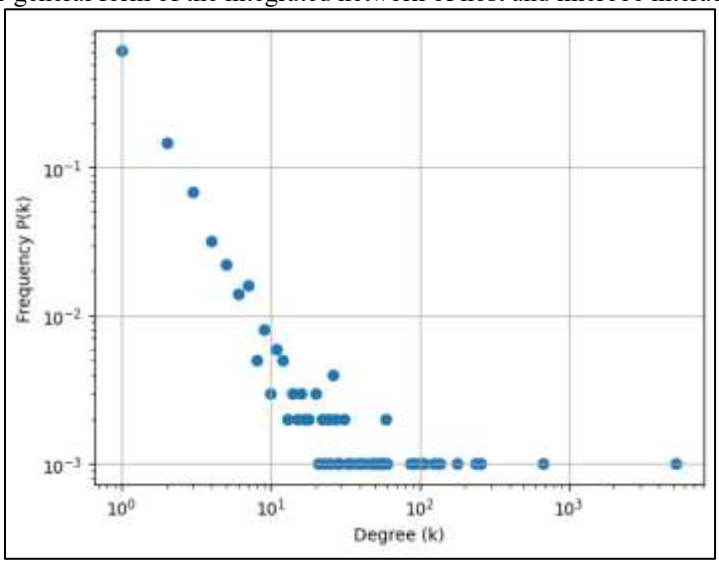

Fig. 3. Degree distribution of the host–microbe interaction network showing scale-free characteristics

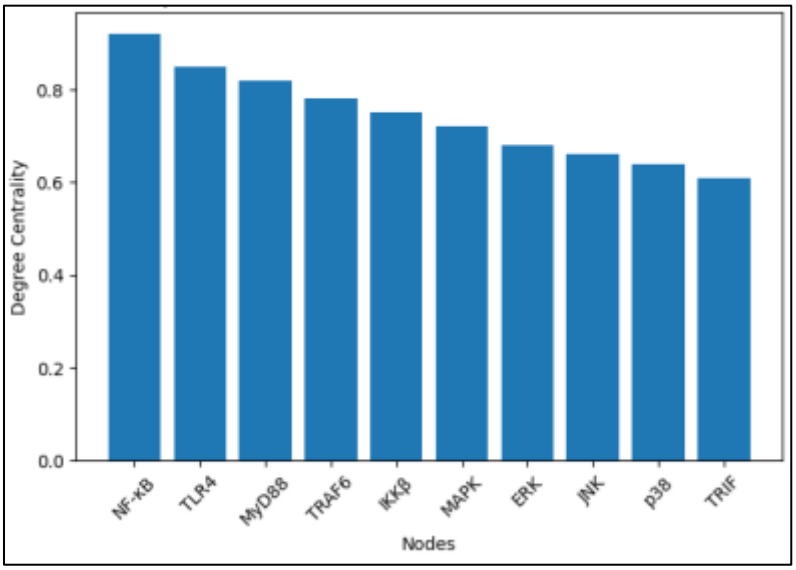

Fig. 4. Top 10 hub nodes identified by degree centrality in the host–microbe interaction network

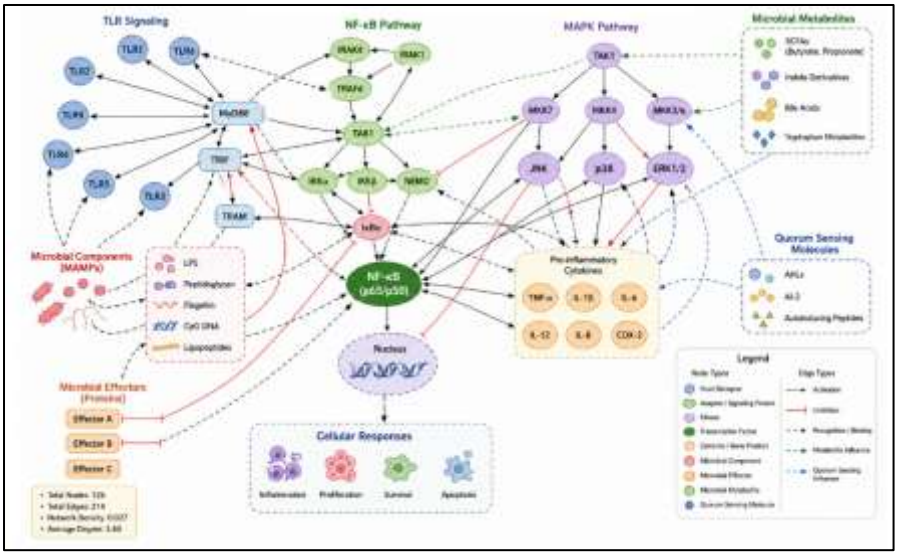

Fig. 5. Visualization of the integrated host–microbe interaction network showing node types (host proteins, microbial components, metabolites) and interaction types (activation, inhibition, and binding).

5. DISCUSSION

The results illustrate the complexity of host microbe interaction networks that is multi-layered with tight integration of molecular signaling pathways and microbial regulatory mechanisms. The designation of NF-KB and MAPK pathways as the hubs is in accordance to prior works which underline its connection to the immune-regulation and inflammatory reactions. (2020)). The current network-level analysis offers a more detailed view of the dynamics of signaling component interactions as compared to the traditional pathway-based studies. Most microbiome research up to date has been done on taxonomic composition or single molecular pathways (Oulas, A., Minadakis, G., Zachariou, M., Sokratous, K., Bourdakou, M. M., and Spyrou, G. M.). (2019) and this article proves the necessity to consider host and microbial systems in the framework of one concept. The high centrality of TLR and NF-kB nodes is consistent with systems biology research which has found these elements to be central regulatory centers in immune signaling networks. Likewise, the modular organization of MAPK signaling pathways reinforces previous observations revealing that these pathways are functional units of bigger signaling systems. Noteworthy, by integrating microbial metabolites with quorum sensing systems, host signaling pathways have been shown to be highly regulated by them. This is in line with recent multi-omics findings which show that molecules of microbial origin can play a crucial role in mediating host physiology and immune functionality (Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Wang, J.). (2010)). Translationalally, the discovery of hub nodes and bottleneck regulators give possible sites of therapeutic intervention. Tampering with critical nodes in the network can be used to modulate disease-related pathways, especially in diseases like inflammatory bowel disease, metabolic, and immune dysregulation.

CONCLUSION AND FUTURE SCOPE

This paper shows a systems-level analysis of hostmicrobe interaction networks combining the molecular signaling pathways, microbial regulatory proteins and networkbased modeling techniques. Using data on KEGG, Reactome and curated bibliography with computational software like Cytoscape, Python (NetworkX) and R (igraph) a detailed interaction network was built and examined. The findings prove that major signaling pathways, especially TLR, NF-0 B and MAPK are major regulatory nodes in the network, integrating immune reactions and cellular adjustments. Scale-free behavior is also identified in the network topology analysis, where highly connected hub nodes like NF-1and TLR4 have an important role in ensuring the stability of the system and cross-talk between host cells and microbial cells. The combination of microbial factors, such as quorum sensing molecules, and host signaling mediators (such as short-chain fatty acids) underscores their important contribution to the regulation of host signaling pathways. The results go beyond the conventional pathway studies by offering a single framework that accounts the bidirectional and dynamic interaction between hosts and microbes. This study provides a better interpretability compared to current methods with network-level insights and key regulatory nodes that have potential therapeutic application. The future research in this area should concentrate on the combination of multi-omics data to improve the solving and accuracy of the interaction networks, and experimental validation of predicted interactions. Moreover, predictive models and

specific therapeutic approaches grounded on network perturbation analysis could be further used to improve the knowledge of microbiome-related diseases and their treatment.

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